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Exploring Genetic Biomarkers in a Sample of Depressed Patients in the UAE

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EXPLORING GENETIC BIOMARKERS IN A SAMPLE OF DEPRESSED PATIENTS IN THE UAE

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Clinical Psychology

Under the Supervision of Dr. Fadwa Al Mughairbi

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Declaration of Original Work

I, Nailah Mahmood, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Exploring Genetic Biomarkers in a Sample of Depressed Patients in the UAE*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Fadwa Al Mughairbi, in the College of Humanities and Social Sciences at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

This study focuses on exploring the expression of genes associated with depressive and anxiety disorders (AD) among depressed individuals. Anxiety and depression are well known to co-occur with each other. Growing research in the field of genetics has indicated moderate familial aggregation for these disorders. The aim of this study is to analyze gene expression among a depressed population residing in the UAE. The genes chosen to be studied (*PPARGC1A*, *CAMKMT*, *HSD11B1*, *SLC6A4* and *MAOA*) have previously been linked to depression and anxiety in other populations. The study employed a case-control design, where gene expression in blood samples of the depressed group (29 participants) were compared with a control group (30 participants). Initial screening of depression levels for all participants was done using the Beck Depression Inventory (BDI) and Patient Health Questionnaire – 9 (PHQ9), and formal diagnosis for participants in the depression group was given by psychiatrists using ICD-10 criteria. Results indicate that the expression of *PPARGC1A* gene is significantly lower among the depressed group. These results indicate a novel association of *PPARGC1A* with depression and open several possibilities for further research to study its role as a protective factor against developing depression.

Keywords: Depression, anxiety, gene expression, *PPARGC1A*, *HSD11B1*, *CAMKMT*, *SLC6A4*.

Title and Abstract (in Arabic)

دراسة العلامات الحيوية الوراثية للمرضى الذين يعانون من الاكتئاب في دولة الامارات العربية المتحدة

الملخص

تركز هذه الدراسة على استكشاف التعبير عن الجينات المرتبطة باضطرابات الاكتئاب والقلق (AD) في عيّنة من الأفراد المصابين بالاكتئاب. من المعروف جيدًا أن القلق والاكتئاب يحدثان مع بعضهما البعض، وقد اشارت البحوث في مجال علم الوراثة إلى وجود تكدس وراثي عائلي معتدل لهذه الاضطرابات. تهدف هذه الدراسة إلى تحليل التعبير الجيني في عيّنة من المصابين بالاكتئاب المقيمين في دولة الإمارات العربية المتحدة. وقد تم مسبقا ربط الجينات التي تم اختيارها في هذه الدراسة (*PPARGC1A*، *CAMKMT*، *HSD11B1*، *SLC6A4* و *MAOA*) بالاكتئاب والقلق في مجموعات أخرى. استخدمت الدراسة تصميمًا للتحكم في الحالات ، حيث تمت مقارنة التعبير الجيني في عينات الدم للاشخاص المصابين الاكتئاب (29 مشاركًا) مع المجموعة الضابطه (30 مشاركًا). تم الفحص الأولي لمستويات الاكتئاب لجميع المشاركين باستخدام استبيان Beck Depression Inventory(BDI) واستبيان صحة المرضى - 9 (PHQ9) ، وتم تقديم التشخيص الرسمي للمشاركين في مجموعة الاكتئاب بواسطة أطباء نفسيين يستخدمون معايير ICD-10. تشير النتائج إلى انخفاض نسبة التعبير الجيني لـ *PPARGC1A* في الافراد المصابين بالاكتئاب . تشير النتائج إلى أن التعبير عن الجين *PPARGC1A* هو أقل بكثير بين المجموعة الاكتئاب. مقارنة بالمجموعة الضابطة. تشير هذه النتائج تشير إلى وجود علاقة جديدة بين *PPARGC1A* والاكتئاب وتفتح العديد من الاحتمالات لمزيد من البحث لدراسة دورها كعامل وقائي ضد الاكتئاب.

مفاهيم البحث الرئيسية: الاكتئاب، القلق، التعبير الجيني، *HSD11B1* ، *CAMKMT*، *SLC6A4*.

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Dedication

To my beloved parent - hope you always remain proud of your girls.

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List of Abbreviations

AD	Anxiety Disorder
CAMKMT	Calmodulin-Lysine N-Methyltransferase
DNA	Deoxyribonucleic Acid
DSM	Diagnostic and Statistical Manual
GAD	Generalized Anxiety Disorder
HSD11B1	11beta-hydroxysteroid dehydrogenase type 1
MAOA	Monoamine Oxidase A
MDD	Major Depressive Disorder
OCD	Obsessive Compulsive Disorder
PD	Panic Disorder
PPARGC1A	PPARG Coactivator 1 Alpha
RNA	Ribonucleic Acid
SAD	Social Anxiety Disorder
SLC6A4	Solute Carrier Family 6 Member 4
SP	Specific Phobia
VATSPSUD	Vietnam Adult Twin Study of Psychiatric and Substance Use Disorders
5-HTTLPR	Serotonin transporter gene – linked polymorphic region

Chapter 1: Introduction

1.1 Overview

1.1.1 Depressive Disorders

Depressive disorders are a group of disorders that are characterized by chronic sadness, feelings of emptiness or irritable mood, along with changes in somatization and cognitions (American Psychiatric Association, 2013). Individuals going through these feelings and changes find it difficult to carry out daily functions. In 2004, the World Health Organization (WHO) reported an estimated 151.2 million individuals to be suffering from a unipolar depressive disorder, making it the most prevalent of mental health conditions (World Health Organization, 2008). For the purposes of their report, severe depression was classed together with active psychosis, severe migraine, quadriplegia, and terminal stage cancer on the basis their disability. Unipolar depressive disorders also accounted for the leading global cause of years lost due to disability (YLD) in both sexes, and in all low-, middle-, and high-income countries. It was also projected to rank first by 2030 in terms of potential years of healthy life lost due to "being in states of poor health or disability" (World Health Organization, 2008).

The various risk factors leading to depression can broadly be categorized as environmental factors and genetic factors. Environmental factors, particularly, abuse and neglect in the childhood years are risk factors for various psychiatric symptoms and disorders, but particularly so for mood and anxiety disorders (Heim, Shugart, Craighead, & Nemeroff, 2010). However, 30-40% of the risk has been found to be genetically determined (Heim & Binder, 2012).

1.1.2 Anxiety Disorders

Anxiety disorders (AD) comprise of a group of disorders that share the common features of excessive fear, anxiety and related behaviors in anticipation of future threats. These include separation anxiety disorder, selective mutism, agoraphobia, generalized anxiety disorder (GAD), social anxiety disorder (SAD), panic disorder (PD) and specific phobia (SP). As opposed to fear, anxiety-related behaviors are often associated with excessive attentiveness in preparation for possible dangers, muscle tension and avoidance or caution (American Psychiatric Association, 2013).

Similar to depression, ADs are more prevalent in women - affecting twice the number of women than men, and often have onset during childhood and adolescence. In fact, individuals within the age range of 10-25 years have been found to be at the highest risk for developing an AD, with more than a third of this population meeting criteria for an AD (Nieto, Patriquin, Nielsen, & Kosten, 2016). Environmental factors are known to be the leading causes of ADs as well. However, recent findings in the field of genetic epidemiology show heritability to be in the range of 30-50%, indicating moderate familial aggregation (Shimada-Sugimoto, Otowa & Hettema, 2015).

1.1.3 Comorbidity of Depression and Anxiety

Major depressive disorder has previously been found to be the most prevalent of all mental health conditions. Though, when taken as a group, the prevalence of mood disorders falls behind ADs (Merikangas et al., 2007). Depression and anxiety are generally seen to co-occur with other psychiatric conditions such as substance use disorders and personality disorders. However, depression comorbid with anxiety can in fact be considered the norm rather than the exception in most clinical presentations

of such disorders (Fava et al., 2008). More than half (57%) of the individuals suffering from a depressive disorder have been found to have a comorbid anxiety disorder (Zimmerman, McDermut, & Mattia, 2000). Anxiety can not only be seen as a comorbidity, but also as a predominant feature of major depressive disorder in some cases (Malhi & Mann, 2018). AD diagnoses in depressive disorders has also been found to increase the likelihood of having suicidal thoughts as well as completed suicide (Goldberg & Fawcett, 2012).

It was found a few decades ago that the comorbidity between depressive and anxiety disorders is not due to chance, and that the occurrence of an affective disorder increases the risk of having an AD and vice-versa (Kessler, 1995). Klein & Riso (1993), and later Neale & Kendler (1995), developed models to explain the comorbidity between these disorders. These models are referred to as KR1-KR11 and NK1-NK12 respectively, all of which are outlined in Table 1 adopted from Neale & Kendler (1995).

Table 1: Klein & Riso and Neale & Kendler's models of comorbidity (Neale & Kendler, 1995)

Model	Cause of comorbidity
KR1/NK1	Comorbidity due to chance
KR2/NK2	Comorbidity due to sampling bias
KR3/NK3	Comorbidity due to population stratification
KR4	Comorbidity due to overlapping diagnostic criteria
KR5	Comorbidity due to one disorder encompassing the other
KR6	Comorbidity due to multiformity of one disorder
KR7	Comorbidity due to heterogeneity
KR8/NK9	Comorbidity due to third independent disorder
KR9/NK4	Comorbidity due to alternative forms or phases
KR10/NK11	Comorbidity due to one disorder being risk factor for the other
KR11/NK10	Comorbidity due to overlapping etiological processes
NK12	Comorbidity due to reciprocal causation

More recently, a review conducted by Middledorp et al. (2005) focusing on twin studies and family studies found most of these models to fit the data (NK6, NK10, NK11 and NK12). Among these models, NK11 (i.e., MDD causing GAD was found

to be the best-fitting model). Their results also ruled out KR8/NK9 as a suitable model. The authors consequently provided different plausible mechanisms for their findings. The first mechanism explains the co-morbidity due to interconnections of different brain regions that are responsible for generating different emotions and responses. The second mechanism uses the heritability of neuroticism as a personality trait as an explanation.

1.1.4 Mental Health in the Arab World

The gulf region has seen rapid change in sociodemographic factors over the past four decades derived by the discovery of oil and the subsequent boost in economy. These changes, which include a shift in family-life, food habits, social cohesion and exercise (Osman & Afifi, 2010), and the drastic generation gap, are bound to cause mental health challenges.

Even as early as in the 1990s, the WHO recommended spending not less than 10% of the country's health care budget on mental health services, reserving 25% of hospital beds for mentally ill patients, as well as having a ratio of 0.25-1.0 psychiatrists for every 10,000 persons (World Health Organization, 1996). In a study published by Okasha (1999), it was reported that the United Arab Emirates had only 30 hospital beds allocated to psychiatric patients and one mental health hospital. There were 40 registered psychiatrists at the time, with a ratio of one psychiatrist per 62,500 individuals in the population. Apart from the psychiatrists, there were 13 psychologists, 30 social workers and 109 psychiatric nurses in the country. However, in a large-scale survey published more than a decade later, the ratio of psychiatrists in the UAE rose from 0.9 to 2 per 100,000 individuals, along with an increase in psychologists and social workers (Okasha, Karam, & Okasha, 2012). This change was

also seen in almost all other Arab nations. Despite the increase in professionals, the budget allocated by governments for mental health services was still found to be far below the recommended amount.

In general, no nation-wide data is currently available on the prevalence of depression in the U.A.E., both for citizens and residents. However, a large-scale study conducted by Moselhy et al. (2012) found that approximately 18.7% of the Emirati population had a then current diagnosis of an anxiety disorder. With regards to research on depression, most of the research conducted focus on studying this disorder in selected small groups of people. For example, prevalence rate of depression was found to be 25.1% among male migrant workers living in the UAE (Al-Maskari et al., 2011), 6-22% among medical residents in the country (Abdulrahman, Farooq, Al Kharmiri, Al Marzooqi, & Carrick, 2018), 10% (post-partum depression) among women in Sharjah (Hamdan & Tamim, 2011), 17.6% among patients with multiple sclerosis (Alsaadi et al., 2017), 63.3% among resident doctors working for Dubai Health Authority (Monsef et al., 2015), and 22.2% among university students (Mellal, Albluwe, & Al-Ashkar, 2014). Monsef et al. (2015) also reported a prevalence rate of 57.4% for anxiety among resident doctors.

1.1.5 Epigenetics

Humans are more alike than different. We are genetically identical in approximately 99% of our genomes, with the 1% difference seen in the form of copy number variations (CNVs), single nucleotide polymorphisms (SNPs) and variation in the number of tandem repeats (Venter et al., 2001). There are several gene x environment (G x E) models that have been used to explain the role of both genes and environment in the development of both depression and anxiety. Of interest is a model

of epigenetics. Epigenetics can be described as the interaction of our genes with our developmental biology, which results in "experience-dependent alteration of epigenetic marks" (Heim & Binder, 2012).. Recent focus of scientific literature on epigenetics shows how different stressors can change the way our genes are expressed, without actually changing the DNA. It has been suggested that environmental factors, such as stress, can affect the epigenetic mechanisms, which in turn may play a role in how these environmental factors contribute towards the development of ADs. These mechanisms have the ability to manipulate or alter an organism's phenotype, and are controlled by chromatic remodeling, DNA methylation, and non-coding RNAs (Nieto, Patriquin, Nielsen, & Kosten, 2016).

Epigenetic mechanisms can occur at any time in one's life. In a remarkable study conducted by McGowan et al. (2009), it was found that childhood abuse induces change in the hypothalamic-pituitary-adrenal (HPA) stress responses and increases the risk of suicide. Furthermore, findings from animal studies suggest a possibility for passing on epigenetic patterns to offspring through multiple mechanisms (Babenko, Kovalchuk, & Metz, 2015). In fact, this has been seen in human studies as well, with prenatal maternal anxiety causing methylation of specific genes in the child (Hompes et al., 2013). Babenko, Kovalchuk, & Metz (2015) proposed that epigenetic alterations caused by prenatal stress, and passed on to offspring, can be considered a powerful influencer of mental health in later stages of the child's life, with possible increased risk of developing mental health disorders such as depression, anxiety and even schizophrenia.

1.2 Statement of Purpose

The purpose of this research is to study the expression of various genes associated with depression and ADs among depressed individuals, in an attempt to identify genetic risk factors in developing depression, and anxiety as a comorbidity. The genes chosen to be studied are *PPARGC1A*, *CAMKMT*, *SLC6A4*, *MAOA* and *HSD11B1*. To the knowledge of the researcher, no such study has been done using the residing population of the United Arab Emirates.

1.3 Literature Review

Genetics play an important role in the formulation of psychiatric disorders. If a group of individuals are exposed to similar adverse life-events, not everyone will develop a similar (or any) psychiatric illness. This is because genetics are at play with how environmental stressors are perceived by the individual, making some more vulnerable to develop psychiatric illnesses than others. For example, McGloin and Widom (2001) found that 48% of children who underwent childhood abuse and neglect did not meet the criteria for a psychiatric disorder.

In the past decade, many researchers have turned toward identifying genetic risk factors associated with various psychiatric conditions, including depression. One such gene is the *SLC6A4*, which codes for the serotonin transporter. One of the regions of this gene is the serotonin transporter promoter polymorphism, *5-HTTLPR*. This polymorphic region has been found to be strongly associated with neuroticism as a personality trait, which is characterized by depression and anxiety along with other negative emotions (Lesch et al., 1996; Sen, Burmeister, & Ghosh, 2004), as well as with the Anxiety factor as measured by Cattell's 16PF personality inventory (Lesch et al., 1996). It has also been found to moderate the relationship between stress and

depression (Karg, Burmeister, Shedden, & Sen, 2011), with individuals with certain allele(s) of this gene being more susceptible to developing and reporting a depressive disorder in response to stressful life events as well as childhood maltreatment (Caspi et al., 2003).

A genome-wide association analysis, carried out by Hettema et al. (2011), on fear-related behaviors in mice and data collected from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD) highlighted the role of the *PPARGC1A* gene as a potential susceptibility gene for anxiety-related disorders out of a list of 52 novel candidate genes. The authors suggest that studying phenotypes related to neuroticism, as a personality trait, in a coordinated manner might be a strong approach towards identifying potential genes related to ADs. This is because twin studies indicate that the genetic factors underlying neuroticism are related to, or overlap with, those that increase susceptibility to various internalizing psychiatric symptoms and disorders, such as depression and anxiety. Since Hettema et al. (2011) claim to be the first to suggest the association of *PPARGC1A* with psychiatric phenotypes, they call for future replication studies to demonstrate this link. In another study, the *PPARGC1A* was studied as a potential susceptibility gene for depression but was found to be unassociated with the disorder during follow-up (Schosser et al., 2011).

In one of the largest and most comprehensive genetic studies, carried out by Otowa et al. (2016) on primary ADs, it was found that the *CAMKMT* gene (along with two other genes) has a significant association with anxiety-phenotypes in a large sample of European ancestry. In animal studies, the CAMKMT gene has been found to play an essential role in the proper functioning of the adult mouse brain, along with normal body growth and somatosensory development (Haziza et al., 2015).

The levels of the monoamine neurotransmitter in the brain is to a large extent regulated by the monoamine oxidase A (MAOA). The MAOA processes monoamine transmitters, serotonin, norepinephrine and dopamine (Naoi, Maruyama, & Shamoto-Nagai, 2008). Serotonin is known to regulate mood in humans. In fact, an effective class of drugs used for the treatment of depression are the MAOA inhibitors (Dannlowski et al., 2009). Dysregulation of the monoamine oxidase A (*MAOA*) gene has been associated with depression among females (Melas et al., 2013). It has also been shown to be significantly associated with PD (Deckert et al., 1999; Maron et al., 2005) and panic attack phenotype (Samochowiec et al., 2004) in females. In a study carried out by Dannlowski et al. (2009), it was observed that carriers of the higher active *MAOA* u-VNTR (upstream variable-number tandem repeat) alleles portrayed a reduction in the amygdala-prefrontal cortex connectivity, which was shown to be significantly associated with acute major depression. A similar significant association between *MAOA* u-VNTR and MDD, especially among females, was also found in other studies (Schulze et al., 2000; Yu et al., 2005)

In an unpublished work conducted in the University of Edinburgh, it was found that the gene *HSD11B1* may be associated with anxiety (Frenken, 2012). The 11beta-hydroxysteroid dehydrogenase Type 1 enzyme, which is associated with the HSD11B1 gene, is responsible for the conversion of cortisone into cortisol (Devang et al., 2017). Cortisol is a "corticosteroid hormone … (that is) secreted into the bloodstream … in response to adrenocorticotrophis hormone (ACTH) … especially in response to stress or injury" (Colman, 2015). The amount of cortisol in body fluids such as blood, saliva or urine is often used as a measure of stress in a person. Prolonged threat perception among anxious individuals may cause their biological stress response system to reduce the secretion of cortisol during the morning period (Miller, Chen, & Zhou, 2007;

O'Donovan et al., 2010). *HSD11B1* has previously been linked to health problems such as insulin resistance, obesity, metabolic syndrome and diabetes. A specific polymorphism of this gene – rs12086634, has also been shown to be positively associated with polycystic ovary syndrome (Devang et al., 2018). In another study, the gene was found to be associated with Alzheimer's disease (de Quervain, 2003).

1.4 Research Hypothesis

Based on past research findings, the following hypotheses were set for this study:

1. The expression of all selected genes is higher among the depressed-group.
2. Expression of *MAOA* is higher among females in the depressed-group.

A higher expression of *PPARGC1A* and *HSD11B1* will provide confirmatory evidence to its association with psychiatric phenotypes.

Chapter 2: Methods

2.1 Participants

Sixty participants were recruited. Thirty participants between the age range of 18-65 were recruited from the outpatient Psychiatric clinic at Al Ain Hospital, Al Ain, for the depressed-group. These participants had a current diagnosis of either MDD, dysthymia or adjustment disorder with depressed features given by a psychiatrist using ICD-10 criteria. Current depressive episode was confirmed using the Beck Depression Inventory - II (BDI-II). Thirty healthy controls between the age range of 18-65 were recruited from UAE University. The mean age of the depressed group was 40.3 and that of the control group was 26.5. Participants in the control-group were excluded if they scored above 13 on the BDI-II or above 9 on the PHQ-9. Participants in the depressed-group were excluded if they had (i) comorbid psychotic features, (ii) comorbid psychiatric disorders such as schizophrenia or personality disorders, or (iii) current substance abuse.

All participants were required to read and sign the informed consent sheet prior to participating in the study. They were also required to complete the Patient Health Questionnaire – 9 (PHQ-9) along with the BDI-II before blood samples were collected. Ethical approval for the study was provided by the research committees at UAE University and Al Ain Hospital.

2.2 Assessments

The Beck Depression Inventory and Patient Health Questionnaire were chosen for this study because its Arabic versions have been validated and found to be reliable (West, 1985; AlHadi et al., 2017). The Patient Health Questionnaire - 9 (PHQ-9) is a

depression module and is a self-administered version of the PRIME-MD diagnostic instrument for common mental disorders (Kroenke, Spitzer, & Williams, 2001). The BDI-II is a 21-item self-report multiple-choice inventory that takes approximately 10 minutes to complete. It is a widely used tool to indicate the severity of depression, available in several languages, and appropriate for use by individuals from 13-80 years of age. The items on this scale are rated on a 4-point scale, with a maximum total score of 63 (Beck, Steer & Brown, 1996). The level of depression based on raw scores for this scale, as provided by the BDI-II Manual is shown is Table 2.

Table 2: BDI-II Scores Interpretation

Raw Scores	Depression Severity
0-13	Indicates minimal depression
14-19	Indicates mild depression
20-28	Indicates moderate depression
29-63	Indicates severe depression

2.3 Blood Sampling

About 2.5 ml of blood sample was collected from each participant in a PAXgene collection-tube provided by Qiagen. These samples were stored in 4°C before extraction. The analysis of all blood samples was carried out at the Behavioral Neuroscience Lab at UAE University.

2.4 RNA Extraction

RNA extraction for all blood samples was done using the PAXgene Blood RNA Kit provided by PreAnalytiX (Qiagen) (Ref #762164).

All blood samples were centrifuged at 5,000 RCF for 10 minutes, after which supernatant was decanted. The remaining pellet was dissolved in RNase free water, and centrifuged for another 10 minutes at 5,000 RCF. Resulting supernatant was discarded and pellet was dissolved in buffer. The entire solution was transferred to a 1.5ml microcentrifuge tube. To this, proteinase K and binding buffer were added and the solution was incubated at 55°C for 10 minutes. The entire lysate was transferred to a shredder column and centrifuged for 3 minutes at 20,000 RCF. The resulting supernatant was transferred to a microcentrifuge tube. Ethanol was added and mixed well, and samples were centrifuged for 2 seconds at 1,000 RCF. 700ul of each sample was transferred to a spin column and centrifuged for 1 minute at 20,000 RCF, after which the old processing tube was replaced with a new one. The rest of the sample was transferred to the spin column, to which buffer BR3 was added. After centrifugation, DNase stock solution and Buffer RDD was added. After incubation, Buffer BR3 was added again and samples were centrifuged for 1 minute at 20,000 RCF. Samples were then washed and eluted.

Once RNA was extracted, qualitative and quantitative reading for all samples was recorded using a spectrophotometer.

2.5 Primers Design

Primers for all the genes were designed using the TaqMan Assays and Arrays tool provided by www.thermofisher.com.

2.6 Conversion of RNA to cDNA

The extracted RNA was converted to single-strand cDNA using Thermo Fisher Scientific High-Capacity cDNA Reverse Transcription Kit (Ref #4368814).

cDNA reaction mixture of 20ul was prepared for each reaction using 2ul 10X RT buffer, 0.8ul 25X dNTP mix, 2ul 10X RT Random Primers, 1ul RT enzyme, and RNA sample with nuclease-free water. The mixture for all samples were loaded on a 96-well plate for RT-PCR.

2.7 Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR)

RT-PCR was carried out using the Thermal Cycler provided by Thermo Fisher Scientific. The following conditions were set up for the plate:

i. Step 1 at 25°C for 10 minutes,
ii. Step 2 at 37°C for 120 minutes,
iii. Step 3 at 85°C for 5 minutes, and
iv. Step 4 at 4°C.

2.8 Quantitative Real Time – Polymerase Chain Reaction (qRT-PCR)

The QuantStudio Real-Time PCR by Thermo Fisher Scientific was used to run the qRT-PCR. A mixture of 10ul was prepared for each reaction using 8ul of master mix containing gene primers and 2ul of 10ng cDNA for each sample, and loaded on a 96-well plate. The following conditions were set-up for the plate:

i. UNG incubation at 50°C for 2 minutes,
ii. Polymerase activation at 95°C for 2 minutes,
iii. PCR denature cycle at 95°C for 3 seconds, and
iv. PCR anneal cycle at 60°C for 30 seconds.

2.9 Statistical Analysis

The Statistical Package for Social Sciences was used for comparative and descriptive analysis of the data.

Chapter 3: Results

The purpose of this study is to explore the genetic biomarkers associated with depression and anxiety disorders in a depressed population by comparing them with a normal (non-depressed) control sample. It was hypothesized that all selected genes will be expressed more in the depressed population. In order to examine the research hypothesis, one sample was excluded from the study based on quantitative analysis of RNA, and fifty-nine samples were used for consequent RT-PCR and qRT-PCR. Results generated from qRT-PCR were subjected to gene expression analysis using the QuantStudio Design and Analysis Software (version 1.3.1).

3.1 Profile and Statistics of Respondents

This study involved two groups of UAE residents. The first group included twenty-nine depressed patients and the second group included 30 non-depressed controls. The distribution of participants between both groups is shown in Table 3.

Table 3: Descriptive Statistics

		Depressed		Control	
		N	%	N	%
Gender	Male	15	51.7%	12	40%
	Female	14	48.3%	18	60%
	Total	29	100%	30	100%
Nationality	Emirati	12	41.4%	15	50%
	Non-Emirati	17	58.6%	15	50%
	Total	29	100%	30	100%

In order to examine the baseline level of depression for both the groups, the independent-samples *t*-test was used (see Table 4). The *t*-test is a statistical procedure that assesses for any statistically significant difference between the means of two groups (Duignan, 2016).

Table 4: Independent Samples *t* Test for Baseline Depression

Group Statistics							
	Group	N	Mean	SD	*t*	df	*p*
BDI	Patient	29	31.90	10.76	12.73	57	.00
	Control	30	5.53	3.53			
PHQ-9	Patient	29	14.34	4.61	13.32	57	.00
	Control	30	2.40	1.65			

3.2 Profile of Extracted RNA

All RNA samples were quantified using a spectrophotometer, and checked for quality. Purity level and concentration of RNA samples is provided in Table 5 (purity level within the range of 1.8 – 2.0 is considered to meet criteria for further analysis).

Table 5: Purity and Concentration of RNA Samples

	Mean Concentration	Mean Purity	
		260/280	260/230
Depressed	149.16	2.04	1.84
Control	140.05	2.04	2.00

3.3 Gene Expression Analysis

Gene expression analysis was carried out by the QuantStudio Design and Analysis Software. Expression level of the genes, when set against a reference level of

1.0 is shown in Figure 1. The independent samples *t* – test was used to check the significance of difference between the expression levels of the genes (Table 6). No significant difference in gene expression was found for the nationality or gender variable within each group, so the data were pooled. The data for expression of *MAOA* between males and females for the depressed-group are shown in Table 7.

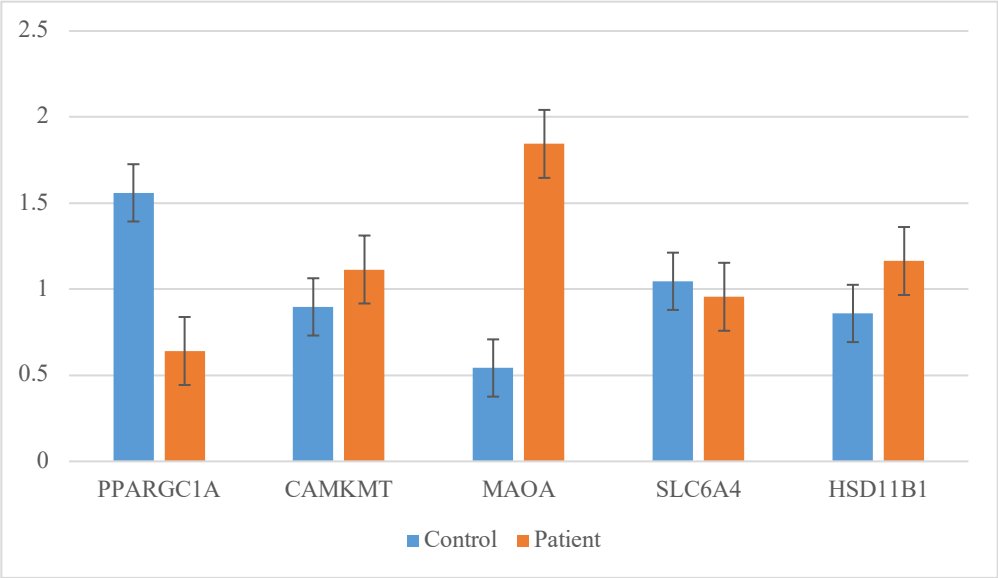

Figure 1: Gene Expression

Table 6: Independent Samples *t* Test for Gene Expression

Group Statistics								
	Group	N	Mean	SD	Mean Difference	*t*	df	*p*
PPARGC1A	Depressed	29	0.45	0.35	0.34	2.39	57	0.02
	Control	30	0.80	0.70				
CAMKMT	Depressed	29	1.35	0.52	-0.17	-1.46	57	0.15
	Control	30	1.18	0.33				
MAOA	Depressed	29	2.05	2.21	-0.98	-1.83	57	0.07
	Control	30	1.07	1.87				
SLC6A4	Depressed	29	1.30	0.90	0.07	0.32	57	0.75
	Control	30	1.37	0.85				
HSD11B1	Depressed	29	1.82	1.18	-0.40	1.71	57	0.10
	Control	30	1.42	0.54				

Table 7: Independent Samples *t* Test for MAOA Gender Comparison

Group Statistics								
	Group	N	Mean	SD	Mean Difference	*t*	df	*p*
MAOA	Male	15	1.75	1.57	0.63	0.75	20	0.47
	Female	14	2.38	2.77				

Chapter 4: Discussion

4.1 Findings

The results obtained from the gene expression analysis provide significant information. Of the five genes selected for analysis, only one of them, i.e. *PPARGC1A*, was seen to have a significant difference between the two groups with $p<0.05$. The *PPARGC1A* gene has previously been shown to be related to anxious-phenotypes in a genome-wide association analysis carried out by Hettema et al. (2011). The results from the current study show that the expression of *PPARGC1A* is significantly higher in the control group as compared to the depressed group. This highlights a novel importance of this gene in association with depression. The results indicate a possible protective role that the gene may play against developing depressive disorders. However, this does not contradict the findings of Hettema et al. (2011) as the sample chosen for this study was that of depression and not anxiety.

PPARGC1A – peroxisome proliferator-activated receptor gamma coactivator 1-alpha – is a transcriptional coactivator for steroid receptors and nuclear receptors. It plays an important role in energy metabolism and is known to be involved in the cellular response to oxidative stress and negative regulation of neuron death. Due to these roles that the gene plays, it could be the case that higher expression of the gene protects individuals from developing a depressive disorder and a decrease in the expression makes them more vulnerable to it. What is especially interesting to note is that *PPARGC1A* is found to be responsive to several forms of environmental stressors including nutritional status and temperature (Puigserver et al., 1998; Scarpulla, 2002) , making it a good candidate for evidence for the epigenetics model. Previous research has found elevated levels of this gene to serve as a protective factor for neural cells

against apoptosis caused by oxidative stress (St-Pierre et al., 2006) and improve neurological symptoms (Tsunemi et al., 2012). Low levels of *PPARGC1A* have also been shown to be associated with Alzheimer's Disease and memory loss (Sweeney & Song, 2016). Since depression is known to precede clinical diagnosis of Alzheimer's Disease, and have a prevalence of upto 50% in patients with Alzheimer's Disease, it is important to study the underlying mechanism of the *PPARGC1A* to better understand its role against depressive disorders.

The difference in expression for the other genes are not significant at p=0.05. This is in contradiction with previous research which have linked these genes to depressive phenotypes. Additionally, even though the expression of the *MAOA* gene has the greatest mean difference (-0.98) between the two groups, the difference is not statistically significant (p = 0.07). This could be due to the large standard deviation (2.21 and 1.87) between the individual values among the samples and the highest standard error mean (0.53) among all the genes. *HSD11B1* was linked to anxiety-phenotypes in a preliminary study (Frenken, 2012). No confirmatory analysis has been conducted to confirm this linkage. The mean difference of *HSD11B1* expression among the two groups is -0.40 and is not statistically significant (p = 0.10). This provides novel information that this gene is not associated with depressive disorders. However, it does reject the results obtained from the preliminary study as no baseline of anxiety levels was collected from the participants.

Independent samples t-test was also run for the *MAOA* gene between both the genders, but no significant difference was seen (p = 0.47). These results were reported as it was hypothesized that the *MAOA* gene will be expressed more among females in the depressed group as compared to the males.

4.2 Limitations

This research has confronted several limitations. Firstly, the biggest limitation faced by this research is the lack of data on current medications taken by the participants, which may impact the expression of genes, such as long-term use of anti-depressants. However, this information was intentionally not collected for the current study to run a basic association analysis for the genes without having to add medications as an additional variable.

Secondly, the nationality variable was kept open which may act as a confounding variable and reduce the significance of the results obtained. Third, baseline data on anxiety levels was not recorded. Lastly, the small sample size may have played a role in the overall results. It has been reported that when conducting research in the field of genetics, a minimum of 5000 cases and 5000 controls would be necessary to obtain 80% power in the data (Shimada-Sugimoto, Otowa & Hettema, 2015). Since such large samples are extremely difficult to recruit for any single study, replications of studies are crucial to confirm any genetic associations discovered.

Chapter 5: Conclusion

This study focused on exploring the expression of genetic bio-markers among depressed patients by comparing them to a non-depressed control group. Five genes were selected for analysis. The genes associated with anxiety were selected due to high comorbidity between depressive and anxiety disorders. Studying these genes among a depressed population would provide insight on the prevalence of anxiety genes among depressed individuals, or, the role of such genes in mood disorders.

Among the five genes analyzed, only the *PPARGC1A* was seen to have a statistically significant difference in the expression levels between the two groups. This result highlights the role that *PPARGC1A* may play in protecting against depressive disorders.

Contrary to previous research, no other significant differences among gene expressions between the two groups were observed in the current study.

5.1 Research Implications

The current study is one of very few studies conducted on genetic association with depression in the region. To the knowledge of the researcher, no such previous study has been published in the country. The results obtained from the study are thus novel and significant. Implementing and making proper use of such results however, will require further research.

Identifying the genes associated with depression can have several implications in any population. With the rise in genetic testing and the boom in genome-wide association studies, it has become easier to test individual biomarkers such as genetic make-up, gene expression, DNA, etc. If depressive genes are identified at a young age, individuals and mental health professionals, and especially family members, can be

cautious about environmental stressors and the kind of support and understanding that is expressed. The results of the current study raise the possibility that *PPARGC1A* could be used as a therapeutic agent in the treatment of depression.

Further research is recommended to confirm the results obtained in the current study. Future studies can focus on the pathways involved in the functioning of *PPARGC1A* to understand its role in depressive disorders. It is also recommended to explore its possible association with the upregulation of key neurotransmitters associated with depression, such as serotonin and dopamine. It is also recommended that further research be conducted keeping the nationality variable constant. A larger sample size can also add significance to future research.

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Appendix

Beck Depression Inventory (English)

Roche | **Beck Depression Inventory** | **Baseline**

V 0477 | CRTN: _____ CRF number: _____ | Page 14 patient Inits: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the one statement in each group that best describes the way you have been feeling during the past two weeks, including today. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
- 0 I do not feel sad.
- 1 I feel sad much of the time.
- 2 I am sad all the time.
- 3 I am so sad or unhappy that I can't stand it.

2. Pessimism
- 0 I am not discouraged about my future.
- 1 I feel more discouraged about my future than I used to be.
- 2 I do not expect things to work out for me.
- 3 I feel my future is hopeless and will only get worse.

3. Past Failure
- 0 I do not feel like a failure.
- 1 I have failed more than I should have.
- 2 As I look back, I see a lot of failures.
- 3 I feel I am a total failure as a person.

4. Loss of Pleasure
- 0 I get as much pleasure as I ever did from the things I enjoy.
- 1 I don't enjoy things as much as I used to.
- 2 I get very little pleasure from the things I used to enjoy.
- 3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
- 0 I don't feel particularly guilty.
- 1 I feel guilty over many things I have done or should have done.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6. Punishment Feelings
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7. Self-Dislike
- 0 I feel the same about myself as ever.
- 1 I have lost confidence in myself.
- 2 I am disappointed in myself.
- 3 I dislike myself.

8. Self-Criticalness
- 0 I don't criticize or blame myself more than usual.
- 1 I am more critical of myself than I used to be.
- 2 I criticize myself for all of my faults.
- 3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10. Crying
- 0 I don't cry anymore than I used to.
- 1 I cry more than I used to.
- 2 I cry over every little thing.
- 3 I feel like crying, but I can't.

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Subtotal Page 1

Continued on Back

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Beck Depression Inventory

CRTN: ____ CRF number: _____ Page 15 patient inits: _____

Baseline

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

3 4 5 6 7 8 9 10 11 12 A B C D E

Subtotal Page 2

Subtotal Page 1

Total Score

NR15645

Beck Depression Inventory (Arabic)

مقياس بيك– II للاكتئاب (BDI-II)

تتضمن هذه القائمة مجموعات من الجمل تصف مشاعرك و أحاسيسك في نواحي متعددة. القيام بقراءة كل مجموعة من الجمل و اختيار جملة واحدة تعطي أدق وصف لحالتك النفسية و للشعور السائد لديك خلال السبعة أيام الماضية بما فيها هذا اليوم. و بعد أن تحدد الجملة التي تصف مشاعرك ضع دائرة حول الرقم الذي يسبقها. أما إذا وجدت في مجموعة ما أن هنالك عدة جمل تنطبق بصورة متساوية على حالتك فضع دائرة حول رقم جملة واحدة منها فقط ترجح أنها الأكثر انطباقا على حالتك

العمر:----------

الجنس: ذكر أنثى

1. الحزن	**6. مشاعر العقاب**
0 أنا لا أشعر بالحزن	0 لا أشعر بأنني أعاقب أو أعذب
1 أشعر بالحزن في أغلب الأوقات	1 أشعر بأنني قد أعاقب أو أعذب
2 أنا حزين في كل الأوقات	2 أتوقع أن أعاقب أو أعذب
3 أنا حزين لدرجة لا أستطيع تحملها	3 أشعر أنني أعاقب أو أعذب الآن
2. التشاؤم	**7. كراهية الذات**
0 أنا لست متشائما تجاه مستقبلي	0 إحساسي تجاه نفسي لم يتغير
1 أشعر بالتشاؤم تجاه مستقبلي أكثر من ذي قبل	1 فقدت الثقة في نفسي
2 لا أتوقع أي مستقبل مشرق	2 أحس بالخيبة تجاه نفسي
3 أشعر بأن مستقبلي بائس و سيكون أكثر سوءا	3 أنا أكره نفسي
3. الفشل السابق	**8. نقد الذات**
0 لا أحس بأنني فاشل	0 لا أنتقد نفسي أو ألومها أكثر من ذي قبل
1 أنا فشلت أكثر مما يجب	1 أنتقد نفسي أكثر من ذي قبل
2 عندما أنظر إلى الماضي أرى الكثير من الفشل	2 ألوم نفسي على كل أخطائي
3 أشعر أنني شخص فاشل تماما	3 ألوم نفسي على كل شئ سيئ حدث
4. فقدان المتعة (اللذة)	**9. الأفكار أو الرغبات الإنتحارية**
0 أتمتع كثيرا كعادتي بالأشياء التي احبها	0 ليست لدي أفكار بقتل نفسي
1 لم أعد أتمتع بالأشياء كما كنت من قبل	1 لدي أفكار بقتل نفسي لكنني لن أنفذها
2 أجد متعة قليلة جدا في الأشياء التي كنت احبها	2 أرغب في قتل نفسي
3 لا أستطيع أن أجد أي متعة في الأشياء التي كنت احبها	3 سوف أقتل نفسي إذا سنحت لي فرصة
5. الشعور بالذنب	**10. البكاء**
0 لا أحس بالذنب	0 لا أبكي أكثر من المعتاد
1 أحس بالذنب تجاه أشياء كثيرة فعلتها أو كان يجب علي أن أفعلها	1 أبكي أكثر من المعتاد
2 أحس بالذنب معظم الأوقات	2 أبكي لأبسط الأسباب
3 أحس بالذنب طوال الوقت	3 أحس برغبة في البكاء لكنني لا أستطيع ذلك

11.عدم الإستقرار أو كثرة الحركة

0 لا أشعر بعدم إستقرار أكثر من المعتاد

1 أشعر بعدم إستقرار أكثر من المعتاد

2 أنا غير مستقر أو كثير الحركة بحيث لا أستطيع البقاء ساكنا

3 أنا غير مستقر لدرجة أنني يجب أن أظل متحركا أو افعل شيئا

12. فقدان الإهتمام

0 لم أفقد الإهتمام (الرغبة) بالآخرين أو النشاطات

1 أنا أقل إهتماما بالآخرين أو النشاطات من ذي قبل

2 فقدت معظم إهتمامي بالآخرين أو الأشياء

3 من الصعب أن أجد رغبة في أي شئ

13. عدم المقدرة علي اتخاذ القرار

0 أتخذ القرارات بنفس الفعالية المعهودة

1 أجد صعوبة في اتخاذ القرارات أكثر من المعهود

2 أجد صعوبة بالغة في اتخاذ القرارات

3 لا أستطيع إتخاذ أي قرار

14. عدم الفائدة (القيمة)

0 لا أشعر بأني عديم الفائدة

1 لاأعتبر نفسي مفيدا و فعالا كما كنت

2 أشعر أنني أقل فائدة من الآخرين

3 أشعر انني عديم الفائدة كلية

15. فقدان الطاقة

0 لدي طاقة أكثر من أي وقت مضي

1 لدي طاقة أقل من المعتاد

2 ليست لدي طاقة كافية لعمل الكثير

3 ليست لدي طاقة كافية لعمل أي شيء

17. التهيج أو حدة الطبع

0 لست متهيجا أكثر من المعتاد

1 أنا متهيج أكثر من المعتاد

2 أنا متهيج أكثر من المعتاد بكثير

3 أنا متهيج طول الوقت

18. تغير الشهية

0 لا يوجد أي تغيير في شهيتي

1أ قلت شهيتي من المعتاد بقليل

1ب زادت شهيتي عن المعتاد بقليل

2أ قلت شهيتي من المعتاد كثيرا

2ب زادت شهيتي عن المعتاد كثيرا

3أ ليست لدي شهية البتة

3ب أكل الطعام كل الوقت

19. صعوبة التركيز

0 أستطيع التركيز أكثر من أي وقت مضي

1 لا أستطيع التركيز بنفس الفعالية المعتادة

2 من الصعب أن اركز علي أي شئ لفترة طويلة

3 لا أستطيع التركيز علي أي شئ

20. التعب أو الفتور

0 لست تعبا أو منهكا أكثر من المعتاد

1 أحس بالتعب أو الفتور أكثر من المعتاد

2 أنا تعب أو منهك لدرجة أنني لا أستطيع عمل الكثير من الأشياء التي كنت أقوم بها

3 أنا تعب أو منهك لدرجة أنني لا أستطيع عمل أغلب الأشياء التي كنت أقوم بها

21. فقدان الرغبة في الجنس

0 لم الاحظ أي تغيير في رغبتي الجنسية حديثا

1 رغبتي في الجنس أقل من المعتاد

2 الآن قلت رغبتي في الجنس كثيرا

3 ليست لدي أي رغبة في الجنس

16. تغيير نمط النوم	
0 لا يوجد أي تغيير في نمط نومي	
1أ أنام أكثر من المعتاد بقليل	
1ب أنام أقل من المعتاد بقليل	
2أ أنام أكثر من المعتاد بكثير	
2ب أنام أقل من المعتاد بكثير	
3أ أنام أغلب اليوم	
3ب أصحو مبكرًا (1–2) ساعة ولا أستطيع النوم ثانية	

Patient Health Questionnaire – 9 (English)

Patient Health Questionnaire (PHQ-9)

Patient name: _____ Date: _____

1. Over the last 2 weeks, how often have you been bothered by any of the following problems?

	Not at all (0)	Several days (1)	More than half the days (2)	Nearly every day (3)
a. Little interest or pleasure in doing things.	☐	☐	☐	☐
b. Feeling down, depressed, or hopeless.	☐	☐	☐	☐
c. Trouble falling/staying asleep, sleeping too much.	☐	☐	☐	☐
d. Feeling tired or having little energy.	☐	☐	☐	☐
e. Poor appetite or overeating.	☐	☐	☐	☐
f. Feeling bad about yourself, or that you are a failure, or have let yourself or your family down.	☐	☐	☐	☐
g. Trouble concentrating on things, such as reading the newspaper or watching TV.	☐	☐	☐	☐
h. Moving or speaking so slowly that other people could have noticed. Or the opposite; being so fidgety or restless that you have been moving around more than usual.	☐	☐	☐	☐
i. Thoughts that you would be better off dead or of hurting yourself in some way.	☐	☐	☐	☐

2. If you checked off any problem on this questionnaire so far, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

☐ Not difficult at all ☐ Somewhat difficult ☐ Very difficult ☐ Extremely difficult

TOTAL SCORE _____

Patient Health Questionnaire – 9 (Arabic)

إسم المريض .....التاريخ : / /

إلى أي مدى كنت منزعجاً خلال الأسبوعين الماضيين بأي مشكلة من المشاكل التالية؟

PHQ-9					
ر	المشكلة	أبدا(0)	عدة ايام	أكثر من نصف الأيام	تقريبا كل يوم(3)
1	لا أهتم ولا أستمتع بعمل أي شيء				
2	الشعور بالكآبة واليأس				
3	صعوبة النوم ، أو النوم بكثرة				
4	الشعور بالتعب وفقدان الهمة				
5	فقدان الشهية ، أو كثرة الأكل				
6	الشعور السيئ تجاه نفسك–أو أنك فاشل أو أنك خيبت أمل اسرتك				
7	صعوبة التركيز ، مثلا اثناء القراءة أو مشاهدة التلفزيون				
8	بطء الحركة أو الحديث لدرجة لا حظها الأخرون ؟ – أو العكس العصبية والتململ والحركة أكثر من المعتاد؟				
9	التفكير في إيذاء نفسك أو أن من الأفضل لك أن تموت				
مجموع الدرجات					